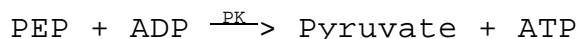


**Enzymatic Assay of UREASE (ATP-Hydrolyzing)
(EC 3.5.1.45)**

PRINCIPLE:



Abbreviations used:

ATP = Adenosine 5'-Triphosphate

UA = Urease, ATP-Hydrolyzing

ADP = Adenosine 5'-Diphosphate

PEP = Phospho(enol)pyruvate

PK = Pyruvate Kinase

NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

LDH = Lactic Dehydrogenase

NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

P_i = Inorganic Phosphate

CONDITIONS: T = 30°C, pH = 8.0, A_{340nm}, Light path = 1.0 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Tris HCl Buffer, pH 8.0 at 30°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 30°C with 1 M HCl.)
- B. 11 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 15 ml in Reagent A using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)
- C. 17 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 15 ml in Reagent A using Phospho(enol)pyruvate, Tri(cyclohexylammonium) Salt, Sigma Prod. No. P-7252.)
- D. 555 mM Potassium Chloride Solution (KCl)
(Prepare 15 ml in Reagent A using Potassium Chloride,

Sigma Prod. No. P-4504.)

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REAGENTS: (continued)

- E. 89 mM Magnesium Sulfate Solution ($MgSO_4$)
(Prepare 15 ml in Reagent A using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)
- F. 85 mM Potassium Bicarbonate Solution ($KHCO_3$)
(Prepare 15 ml in Reagent A using Potassium Bicarbonate, Sigma Prod. NO. P-9144.)
- G. 4.5 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Prepare 1 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129.)
- H. 300 mM Urea Solution (Urea)
(Prepare 10 ml in deionized water using Urea, Sigma Prod. No. U-5128.)
- I. PK/LD Enzymes Suspension¹ (PK/LDH)
(Use PK/LDH Enzymes Suspension, Sigma Stock No. 40-7.)
- J. 50 mM Tris HCl, pH 8.0 at 30°C (Enzyme Diluent)
(Prepare 50 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 30°C with 1 M HCl.)
- K. Urease (ATP-Hydrolyzing) Enzyme Solution (UA)
(Immediately before use, prepare a solution containing 0.2 - 0.4 unit/ml of Urease (ATP-Hydrolyzing) in cold Reagent J.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable containers:

Reagent A (Buffer)	49.50
Reagent B (ATP)	10.00
Reagent C (PEP)	10.00
Reagent D (KCl)	10.00
Reagent E ($MgSO_4$)	10.00
Reagent F ($KHCO_3$)	10.00
Reagent I (PK/LDH)	0.50

Mix by swirling.

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PROCEDURE: (continued)

Pipette (in milliliters) the following reagents into suitable cuvettes:

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	2.70	2.70
Reagent G (β -NADH)	0.10	0.10
Reagent H (Urea)	0.10	0.10

Mix by inversion and equilibrate to 30°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent K (UA)	0.10	-----
Reagent J (Enzyme Diluent)	-----	0.10

Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank}}{(6.22) (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of β -NADH at 340 nm

RM = Reaction Mix

UNIT DEFINITION:

One unit will liberate 2 μmoles of NH_3 from 1 μmole of urea per minute at pH 8.0 at 30°C in a coupled system with PK/LDH.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 91 mM Tris, 1.0 mM ATP, 1.5 mM phospho(enol)pyruvate, 50 mM potassium chloride, 8.0 mM magnesium sulfate, 7.7 mM potassium bicarbonate, 0.15 mM β -NADH, 10 mM urea, 3.2 units pyruvate kinase, 4.5 units lactic dehydrogenase, and 0.02 - 0.04 unit urease (ATP-hydrolyzing).

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REFERENCE:

Roon, R. J. and Levenberg, B. (1970) *Methods in Enzymology*, Volume XVII, Part A, 317-324.

NOTES:

1. Contains not less than 700 Pyruvate Kinase units and 1000 Lactic Dehydrogenase units per ml.
2. Unit Definition for L-Lactic Dehydrogenase: One unit will reduce 1.0 μ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
3. Unit Definition for Pyruvate Kinase: One unit will convert 1.0 μ mole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.
4. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.